

[illegible]

Conf: 2164
Group: 1651
Examiner: Ruth A. Davis

DECLARATION UNDER RULE 132

Sir:

1. I am a citizen of the Netherlands,
2. My educational and technical background in the field of Biology is as follows:
 - a) I am a PhD graduate from 1995;
 - b) From 1995 till 1996, I was employed at Dept. of Behavioural Biology, Institute for Toxicology, ETH Zürich, Schwerzenbach, Switzerland;
 - c) From 1996 till 1999, I was employed at Netherlands Institute for Brain Research,

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Amsterdam, The Netherlands;

d) I have been employed by Nutricia N.V. since 2000, presently as a senior scientist dealing with nutrition and neuropsychological disorders.

3. I have read Kiliaan et al. US application 09/703,798 filed November 2, 2001;
4. I make this declaration in support of the present application, and to provide evidence demonstrating that one of ordinary skill in the art would not find the presently-claimed invention obvious in view of several publications cited in the Office Action mailed on November 17, 2004.

In the Office Action, claims 56, 59 – 62, 65, 69 – 70 and 72 were rejected under 35 USC §103(a) as allegedly being unpatentable over HORROBIN 4,810,497, DELLA VALLE et al. 4,595,680 and FUGH-BERMAN et al.

Claims 56 – 58 were rejected under 35 USC §103(a) as allegedly being unpatentable over HORROBIN, DELLA VALLE et al., FUGH-BERMAN et al. and TAIYO FISHERY CO., LTD.

Claim 63 was rejected under 35 USC §103(a) as allegedly being unpatentable over HORROBIN, DELLA VALLE et al., FUGH-BERMAN et al. and YU et al. 5,177,082.

Claims 64 – 65 were rejected under 35 USC §103(a) as allegedly being unpatentable over HORROBIN, DELLA VALLE et al., FUGH-BERMAN et al. and SMITH et al. 6,008,221.

Claims 65 – 66 were rejected under 35 USC §103(a) as allegedly being unpatentable over HORROBIN, DELLA VALLE et al., FUGH-BERMAN et al. and HUTTERER 4,837,219.

Claim 71 was rejected under 35 USC §103(a) as allegedly being unpatentable over HORROBIN, DELLA VALLE et al., FUGH-BERMAN et al., SMITH et al., HUTTERER and GLICK 5,004,615.

Claims 67 – 68 were rejected under 35 USC §103(a) as allegedly being unpatentable over HORROBIN, DELLA VALLE et al., FUGH-BERMAN et al. and RABIEN (DE 4309217).

After reviewing the Office Action, I do not believe that any of the proposed combinations of publications disclose or suggest the claimed invention. The

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publications, alone or in combination with each other, simply do not teach the recited combination of components or ratios in the pending claims. In addition, I do not believe that any of the proposed combination of references would lead to the unexpected results exhibited by the claimed invention shown in the experimental data discussed below:

EXPERIMENTAL DATA

Introduction

Acetylcholine is a neurotransmitter in the brain, which is essential for normal cognitive functioning. In dementias like Alzheimer's disease (AD), there is a loss of cholinergic neurons and their fibres innervating the cortical projection areas that are involved in cognitive processes. One kind of pharmacotherapy that is often applied to AD patients is the administration of acetyl-cholinesterase inhibitors. These drugs inhibit the breakdown of acetylcholine and thereby prolong the action of reduced levels of the transmitter on its cholinergic receptors in the brains of the demented patients.

The declaration submitted September 15, 2004 contained data indicating that the combined administration of specific fatty acids and phospholipids results in an increased binding of pharmacological agents to G-protein coupled receptors, including muscarinic acetylcholine receptors.

We now performed an in vitro study to examine the effects of fatty acid and phospholipid supplementation on cholinergic receptor activation. Beta TC-6 cells expressing the G-protein coupled muscarinic M1 receptor were used after 24 hours of supplementation. Oxotremorine, a pharmacological agent that acts as an agonist of muscarinic acetylcholine receptors, was used to activate the M1 receptors. Receptor activation and the resulting difference in membrane potential were measured as a change in fluorescent signal in a Flex Station.

Experimental details

Beta TC-6 cells (mouse pancreas) were cultured in a 96 wells plate, with 3×10^6 100 μ l/well. Supplementation of cells was achieved by replacing the standard medium with a medium containing additional fatty acids (40 or 60 μ M) and/or phospholipids (5 or 10 μ M). After 24 hours of supplementation, Oxotremorine-induced muscarinic M1 receptor activity in the cells was measured in a Flex Station II-384 (Molecular Devices).

For the measurements in the Flex Station, medium was removed from the cells and replaced by 25 μ l HBSS + 50 μ l Membrane potential reagent (Molecular Devices), followed by 30 min incubation at room temperature.

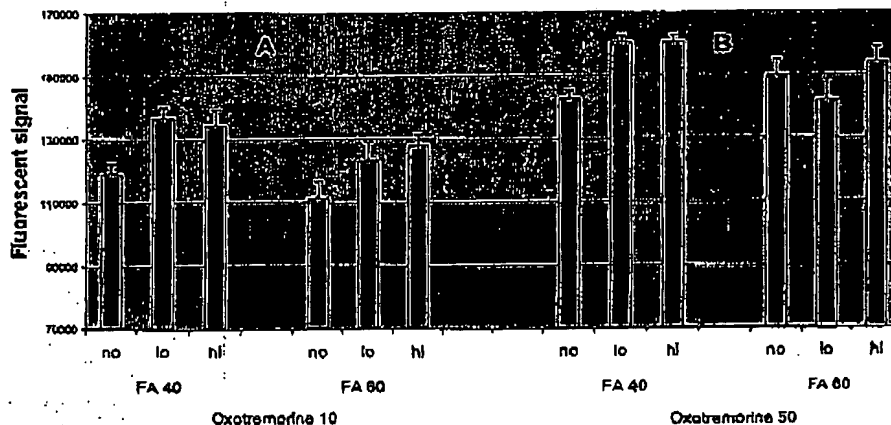
Fluorescent measurements of membrane potentials in the flex station were made at wavelengths of Ex: 530 and Em: 565. During the measurements, either 10 or 50 μ M of the M1 receptor agonist Oxotremorine was added to the cells. Receptor activation and the resultant difference in membrane potential were measured as a change in fluorescent signal.

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Results

The results (in terms of fluorescence signal) obtained for media containing 40 and 60 μM fatty acids (FA) are shown in figure 1, where "no", "lo" and "hi" stand for the amounts of phospholipids that were supplemented, i.e. 0, 5 and 10 μM , respectively. Figure 1a (left) shows the results for 10 μM Oxotremorine, figure 1b (right) for 50 μM Oxotremorine.



An overall analysis of the data indicates that the fluorescent signal that was measured after supplementation, depended on the concentrations of Oxotremorine ($F(1,150)=78.92$, $p<0.001$) and phospholipids ($F(2,150)=6.11$, $p<0.005$). The M1 receptor activation by 50 μM of Oxotremorine (figure B) was stronger than the activation by 10 μM of Oxotremorine (figure A). The addition of phospholipids to the medium containing fatty acids resulted in stronger receptor activation, where both the 5 ("lo") and the 10 μM ("hi") concentration resulted in a significantly increased signal as compared to the 0 μM ("no") concentration ($p<0.01$ and $p<0.001$, respectively).

Discussion & conclusion

The present data show that Oxotremorine induces a dose-dependent activation of muscarinic acetylcholine receptors expressed by Beta TC-6 cells. The signal induced by Oxotremorine is specifically enhanced when, on top of supplementation of the cells with fatty acids, phospholipids are also added.

Taken together, the present data show that especially the combined supplementation of fatty acids and phospholipids results in an optimal response of an agonist at the G-protein coupled M1 acetylcholine receptor. Such supplementation can therefore also be expected to improve receptor activation by the naturally occurring agonist, the neurotransmitter acetylcholine. In this way, combined supplementation of fatty acids and phospholipids may compensate for the loss of acetylcholine in dementia and support normal cognitive functioning in dementia.

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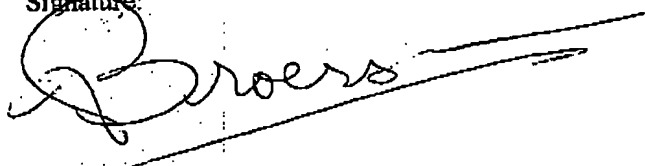
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Thus, in view of the unexpected results exhibited by the claimed invention and deficiencies of the cited publications, I declare that one skilled in the art would not find that any of the proposed combinations render obvious the claimed invention.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: Wageningen May 11, 2005
 (place) (date)

Signature:

A handwritten signature in cursive script, appearing to read "Broers", is written over a horizontal line.

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